Biochip for DNA Amplification and Label-free DNA Detection

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Summary

A silicon-glass based DNA micro analysis system is demonstrated. This biochip is able to amplify and detect specific DNA. First a PCR is run in a micro chamber to generate DNA copies from a specific target gene (16 rRNA). The PCR solution is pumped via a simple micro channel to a capacitive DNA sensing area where target DNA hybridisation is detected. The sensor consists of functionalized nanoscaled interdigitated electrodes for label-free DNA recognition. This total biochemical analysis system is able to detect pathogenic bacteria in a miniaturized, cost effective and fast way.

Motivation

In the field of on-chip (bio)chemical analysis systems, a lot of effort is put into the development of integrated microfluidic systems for analyzing DNA. Such micro scale devices allow point-of-care tests that are less expensive and faster than conventional laboratory analysis. The biochips are able to amplify and to detect DNA. In literature different bioanalytical microsystems have been described[1]. To achieve an easy-to-use and cheap handheld device optical detection methods are replaced by the label-free capacitive DNA sensing[2]. In our contribution we demonstrate the implementation of a hybrid analysis system. The DNA amplification and DNA detection occurs in separated chambers which are connected via a simple fluidic channel. This hybrid system ensures cheap chip production and results in high fabrication yield.

Results

The silicon-glass based DNA analysis system is fabricated using photolithography. The PCR (polymerase chain reaction) chamber for DNA amplification consists of a cavity in silicon (using deep reactive ion etching) treated with thermal oxide for biocompatibility and an anodically bonded glass cover. The access holes are drilled in glass (Fig. 1). The functionality of the PCR chip has been tested by placing it into a conventional thermocycler using a standard PCR program. In Fig. 2 the result of a gel electrophoresis is depicted, where the PCR amplification in the chip is compared to that in conventional PCR reaction tubes. The on-chip PCR is almost as good as the classical method. To achieve a standalone PCR system the thermocycler is replaced by a heater setup, where a commercially available thermofoil heater and a Pt100 sensor are used. The temperature cycles are controlled by a self-created LabVIEW program. For detecting label-free DNA a capacitive sensor has been fabricated (Fig. 3). The capacitor is formed by interdigitated electrodes (IDE) made of gold with 500nm line-space on a silicon-dioxide substrate and the medium between them. The parameter of interest is the capacitance which changes when the specific DNA strands (result of the PCR) hybridize on the DNA probes (covalent bond on the IDE). Fig. 4 shows preliminary measurements of the DNA sensor in hybridisation buffer (4x SSC, 0.1% SDS) between two functionalized IDEs with different ssDNA (sep2 and eco3). The measured capacitance difference between the two ssDNA amounts to 10%. A capacitance change in this range and higher will be expected after DNA hybridization (DNA amount increases). In the next step, the two subsystems will be combined to get a single biochemical analysis system (Fig. 5). In this system, the amplified DNA will be pumped from the PCR chamber via a fluidic channel over an access port into the hollow cylinder where the DNA detection takes place. This presented biochip is suitable for cost-effective and fast detection of pathogenic bacteria.

[1] T. M.-H. Lee, I-M. Hsing, "DNA-based bioanalytical microsystems for handheld device applications", Analytica Chimica Acta, vol. 556, p. 26, 2006.

[2] P. Van Gerwen, et al., "Nanoscaled interdigitated electrode arrays for biochemical sensors", Sens. Actuators B, vol. 49, p. 73, 1998.

Figures

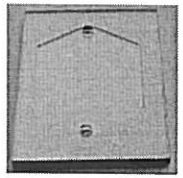

Fig. 1: Photograph of the fabricated PCR chip; Dimensions of the chip: 15 x 20 x 1 mm³ with a reaction volume of about 25 µl; Diameter of the access holes: 1 mm

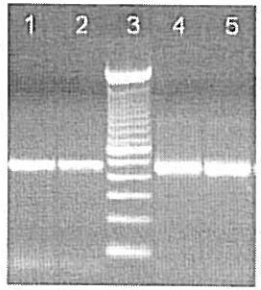

Fig. 2: Agarose gel electrophoresis of 16S rRNA PCR product (497 bp) amplified from E-coli DNA; PCR chip (Lane 1 and2) in comparison to conventional PCR reaction tubes (Lane 4 and 5); Lane 3: 100 bp ladder.

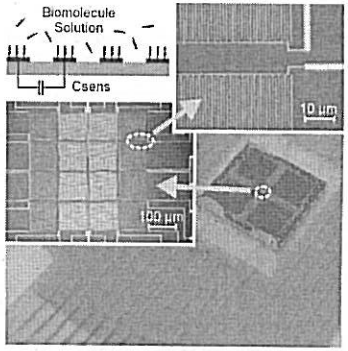

Fig. 3: Electrode chip bonded on a PCB: On the chip (size: 8 x 8 mm²) are 16 interdigitated gold electrodes integrated with a line-space distance of 500 nm. The IDEs are functionalized with different bacterial-species specific DNA. Scheme (top left) illustrates the capacitive sensor principle.

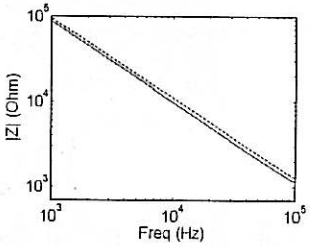

Fig. 4: Measured impedance of the DNA sensor: Electrodes functionalized with 39 base long ssDNA called eco3 (dotted line) and electrodes functionalized with 24 base long ssDNA called sep2 (solid line).

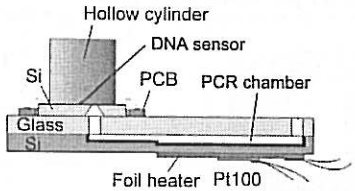

Fig. 5: Schematic of the total microsystem for DNA amplification and label-free DNA detection.